2757-Pos Board B527

A Cardiac Specific Inducible Mouse Model of Timothy Syndrome 2

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Timothy syndrome (TS) is an extremely rare human disorder resulting from a single point mutation (G406R) in the intracellular part of the S6 transmembrane segment of domain 1 of Cav1.2. This region is encoded in a mutually exclusive manner by exons 8/8a.

The identical glycine to arginine mutation occurs in both the 8a and 8 human splice variants resulting in TS1 and TS2. Heterozygous expression of the TS2 defect is lethal to mice. However, the TS2 defect is tolerated when suppressed though transcriptional interference by the NEO promoter (TS2-NEO mouse). By crossing TS2-NEO with a cardiac-specific tamoxifen-inducible CRE we can upregulate expression of the mutant TS2 calcium channel.

We extracted mRNA from cardiac left ventricle and probed with PCR primers to exon 7 and exon 9. Individual PCR products were incorporated into plasmid DNA and individual clones were isolated. This method detected exon 8a:8 ratios in normal mice as 0:58 and 1:56 in TS2-NEO mice suggesting very low level expression of the exon related to TS1. Only 1 in 56 TS2-NEO colonies expressed mutant channels in heart suggesting that the NEO cassette effectively supressed mutant exon 8 isoforms. TS2-NEO mice were crossed with a cardiac specific promoter regulating a CRE recombinase which can be induced with tamoxifen. When activated with tamoxifen (to remove the NEO cassette and suppression of the TS2 mutation) the ratio of mutant to non-mutant exon increased from 1:34 to 29:72. Removal of the NEO cassette induced a strong slowing of inactivation in isolated myocytes. Tamoxifen-induced removal of suppression of the TS2 mutant exon increased QT duration from 33.1 ± 1.8 ms to 74.0 ± 5.5 ms ($n=5, p<0.01$). These results demonstrate the critical role of the L-type calcium channel inactivation in repolarization.

2758-Pos Board B528

Nonlinear Threshold Behavior in the Induction of Arrhythmias by Channels Bearing Timothy Syndrome Mutations

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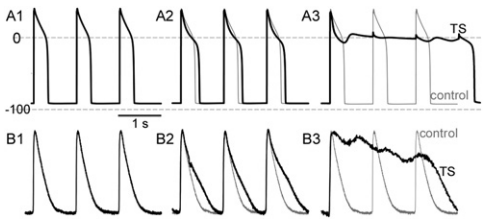
Timothy Syndrome (TS) is a multisystem disorder, featuring cardiac action-potential prolongation with paroxysms of life-threatening arrhythmias. The underlying defect is a single point mutation in $Ca_V1.2$ channels. Interestingly, recent clinical studies hint that overt disease manifestation might be mitigated by diminished expression of mutant channels. To gain insight, we undertook in-depth biophysical characterization of TS channels, and used this profile to refine a widely-utilized ventricular myocyte model (*AJP* **292**:H2854). Simulations of variable levels of TS channels suggested a remarkable behavior. As expected, a modest increase in TS channels produced moderate action-potential prolongation (**A1,** control; **A2,** TS). By contrast, only a minute further increase in TS channels induced flagrant disturbances of excitability (**A3**), suggesting a nonlinear threshold for induction of frank arrhythmias. To explore this experimentally in cell networks, we variably expressed TS channels in monolayers of cultured guinea-pig ventricular myocytes. Indeed, conservative expression of TS channels yielded graded action-potential prolongation (**B1,** control; **B2,** TS), but small further elevation begat severe disturbances (**B3**). If like behavior exists in the heart, limited changes in TS expression may generate dramatic alterations in arrhythmogenic potential, an important principle for therapeutics.



2759-Pos Board B529

Effect of Acidosis on Ventricular L-Type Calcium Current during Action Potentials

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Changes in extra- and intracellular pH (pH_o and pH_i) accompany myocardial ischemia and protons have major effects on ventricular ion currents. However, the separate action of low pH_o and low pH_i on L-type Ca^{2+} current ($I_{Ca,L}$) during action potentials is unresolved. $I_{Ca,L}$ was measured (at 37°C) by applying action potential voltage-clamps (AP-clamp) to adult rabbit ventricular myocytes bathed in CO_2/HCO_3^--free solution, with sarcolemmal sodium-hydrogen exchange blocked (30μM cariporide). pH_i and $Ca^{2+}{}_i$ were measured with SNARF-1 and fluo-4, respectively. Starting from control conditions (pH_o 7.4; pH_i 7.2) each type of acidosis was applied for 2 min: low pH_o (pH_o 6.5, pH_i 7.1), low pH_i (pH_o 7.4; pH_i 6.7, achieved with 80mM acetate superfusion) and combined acidosis (pH_o 6.5; pH_i 6.7). In current-clamped cells,low pH_o shortened AP duration, whereas low pH_i or combined acidosis lengthened AP duration and slowed phase 1 repolarization. During AP-clamps, initial peak $I_{Ca,L}$ was reduced by each type of acidosis. For low pH_o, this effect was mediated largely by direct channel pore block. For intracellular and combined acidosis, it resulted largely from: a) increased steady-state inactivation of $I_{Ca,L}$ produced by an $H^+{}_i$-induced rise in diastolic $Ca^{2+}{}_i$ and b) by slowing of phase 1 repolarization. Net Ca^{2+} influx via $I_{Ca,L}$ was reduced by low pH_o but markedly increased by low pH_i or combined acidosis, the latter two effects mediated by proton-induced slowing of $I_{Ca,L}$ inactivation kinetics. Whole-cell modeling successfully predicted the AP changes induced by low pH_o and low pH_i plus many of the accompanying changes in Ca^{2+} signaling. We conclude that the pH_i-versus-pH_o control of $I_{Ca,L}$ will exert a major influence on electrical and Ca^{2+}-dependent signaling during acid-base disturbances in the heart.

2760-Pos Board B530

Effects of Anandamide on Electrical Activity, Calcium Transients and Contraction in Guinea Pig Cardiac Ventricular Myocytes

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Anandamide is an endocannabinoid which reduces cardiac contractility. Although the effects of endocannabinoids are generally attributed to their association with G-protein-coupled cannabinoid CB_1 and CB_2 receptors, the identity of the receptor(s) or target(s) responsible for the effects of anandamide in the heart are unknown.

Anandamide (10 μM) reduced action potential (AP) duration and contraction in guinea pig ventricular myocytes stimulated to fire APs using an intracellular microelectrode. In addition, anandamide reduced peak amplitude of L-type Ca^{2+} currents over the range −30 to + 60 mV (switched voltage clamp, step depolarisations from −40 mV), with a reduction in peak current at 0 mV of 57 ± 11 % ($n=6, p<0.01$). Ca^{2+} transients measured in field-stimulated cells using Fluo-5F were also reduced. The reduction in AP duration in response to anandamide was partially inhibited by the CB_2 receptor antagonist AM 630 (1 μM), but effects appeared non-competitive, and were smaller than predicted based on a K_i value of 31 nM for CB_2 receptors. The CB_1 receptor antagonist AM 281 (1 μM), the $TRPV_1$ receptor antagonist capsazepine (1 μM), and O-1918 (an antagonist of a reported non CB_1/non CB_2 cannabinoid receptor; 1 μM) failed to inhibit responses to 10 μM anandamide. Furthermore, the selective CB_1 receptor agonist ACEA, and the selective CB_2 receptor agonist HU-308 (both 10 μM) were without significant effect on AP duration.

The effects of anandamide can, at least in part, be accounted for by an inhibition of L-type Ca^{2+} currents. However, additional effects on other ion channels or intracellular targets cannot be ruled out. It appears that if a cannabinoid receptor mediates these effects, the characteristics of this receptor are not those expected of conventional CB_1 or CB_2 receptors.

2761-Pos Board B531

The Role of Caveolae and Membrane Conformation in Load-Induced Ventricular Conduction Slowing

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Mechanical load has been shown to alter cardiac electrophysiology, but the underlying mechanisms are still not completely understood. Conduction slowing has been observed during increased ventricular load in the isolated heart and has been associated with changes in intercellular coupling and membrane capacitance. The goal of this work was to investigate the effect of increased ventricular pressure on cardiomyocyte membrane conformation and caveolar structure, and the role of these changes in conduction slowing associated with load. Murine hearts were isolated, retrogradely perfused, and pressure loaded through a cannula inserted into the left ventricular cavity. In the pressure loaded wild-type heart, electron microscopy revealed a tautening of excess membrane folds, fewer sub-sarcolemmal caveolae, and an apparent opening of the neck of sarcolemmal caveolae compared to the unloaded state. Optical mapping revealed a significant 15-18% slowing of conduction velocity with load in both the directions of fastest and slowest conduction (CVmax and CVmin, respectively). Hearts isolated from caveolin-3 deficient mice have no caveolae on the cardiomyocyte membranes, and control hearts treated with methyl-β-cyclodextrin (MβCD) have a diminished number of caveolar structures, as assessed by electron microscopy. These hearts lacked changes in